



CERTIFIED REFERENCE MATERIAL BCR[®] – 507R

CERTIFICATE OF ANALYSIS

SALMONELLA TYPHIMURIUM IN MILK POWDER			
Colony forming particles of <i>Salmonella typhimurium</i> according to the procedure	Number of colony forming particles (cfp)		Number of accepted sets of data p
	Certified value ¹⁾ [cfp/capsule]	Uncertainty interval ²⁾ [cfp/capsule]	
Enumeration procedure	5.0	4.5 - 5.4	10
Fraction of negative capsules of <i>Salmonella typhimurium</i> according to the procedure	Fraction of negative capsules		Number of accepted sets of data p
	Certified value ⁴⁾ [%]	Uncertainty interval ³⁾ [%]	
Enumeration procedure	1.1	0 - 2.1	10
Presence/absence procedure	1.6	0 - 2.8	11
<p>1) This certified value is the unweighted mean of the total number of 520 accepted capsule counts, independently obtained from 10 laboratories. The certified value is traceable to the respective procedure used (see annex D of the report).</p> <p>2) This value is the two-sided 95% confidence interval, associated with the certified values of the respective procedure used.</p> <p>3) These values are the one-sided 95% confidence interval, associated with the certified values of the respective procedure used.</p> <p>4) Fraction of capsules in which no <i>Salmonella</i> could be detected.</p>			

This certificate is valid for one year after purchase.

Sales date:

The minimum amount of sample to be used is the entire capsule.

NOTE

This material has been certified by BCR (Community Bureau of Reference, the former reference materials programme of the European Commission). The certificate has been revised under the responsibility of IRMM.

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Signed: _____

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DESCRIPTION OF THE SAMPLE

The CRM consists of 0.29 g artificially contaminated spray dried milk contained in a blue/white gelatine capsule. The strain used for the contamination is *Salmonella typhimurium*. The capsules are packed in a plastic container with a silicagel desiccant bag.

ANALYTICAL METHOD USED FOR CERTIFICATION

- a. ISO 6579 standard for the detection of *Salmonella* (Anonymous, 1993) as described in annex F of the certification report (presence/absence procedure).
- b. A specifically developed enumeration procedure of *Salmonella*, as described in annex D of the certification report (enumeration procedure).

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SAFETY INFORMATION

The usual laboratory safety precautions for Biosafety level II pathogens apply.

This CRM is intended for in vitro analysis only. **DO NOT TOUCH THE CAPSULES BY HAND, USE STERILE FORCEPS OR WEAR STERILE GLOVES. THE CAPSULES SHOULD NOT BE OPENED.**

INSTRUCTIONS FOR USE

Presence/absence of *Salmonella*

The material is intended for the evaluation of the performance of the ISO 6579 standard for the detection of *Salmonella* (Anonymous, 1993).

Each capsule is aseptically added **as a whole** to a jar containing the enrichment broth. The broth must be between room temperature and 38 °C before a capsule is added. The jars are handled further according to the ISO standard.

Table 1 presents the recommended number of capsules to be examined and the minimum number of capsules to be found positive for *Salmonella*, in relation to the fraction of negatives a laboratory works with (called π_{lab}) for the certified fraction of 1.6 %. A laboratory has to choose which π_{lab} it wants to be able to detect. The more capsules that can be examined the better the π_{lab} can be tested against the certified fraction of negatives. A laboratory has to find an optimum between the π_{lab} it wants to be able to detect and the number of capsules that it can examine at the same time.

For example: a laboratory wants to be able to detect a π_{lab} of 10 %. Using Table 1 the laboratory needs to examine 42 capsules at one time. Finding 39 positives or less out of the 42 capsules examined indicates that the laboratory finds more negatives than can be expected (based on the certified value and the number of capsules examined). Finding 40, 41 or 42 positives indicates that there is no reason to assume that the laboratory works with a π_{lab} that deviates from the certified fraction of negatives (remember that only a deviation from π_{lab} of 10 % or more could be detected in this case).

More details on the recommendations for the number of capsules to be examined are presented in annex J of the report and by Van Dommelen (1995). The method for the calculation of the minimum number of capsules to be found positive for *Salmonella* is presented in annex K of the report.

Note:

- The performance of the presence/absence procedure for *Salmonella* as described above does not test the suppression of *Salmonella* by competitive microorganisms in the procedure. To do so the

reference materials need to be analysed in combination with (food) samples. This can be done by adding a capsule to the homogenised (food) sample. The number of *Salmonella* isolations obtained will in most cases be lower than could be expected according to the calculated number. Also each (food) sample investigated should be checked for the presence of *Salmonella*. The use of the reference materials added to food samples is described in more detail by In 't Veld and Notermans (1992).

- To compare procedures for the detection of *Salmonella* using a similar (pre)enrichment broth as first enrichment step, it is advisable to use this joint (pre)enrichment broth for the inoculation of the various selective enrichment broths to be evaluated. If one of the procedures applied obtains a positive *Salmonella* isolation (meaning that the reference sample used was contaminated) all procedures should obtain a positive *Salmonella* isolation. The overall performance (number of samples found positive with at least one procedure) should conform to the calculated number of *Salmonella* isolations.

Table 1: Recommended number of capsules and minimum number of capsules to be found positive for *Salmonella* to be able to detect a certain laboratory fraction of negatives (π_{lab}) with a power of 0.80, for the certified fraction of 1.6 %.

π_{lab}	recommended number of capsules to be examined	minimum number of capsules to be found positive for <i>Salmonella</i>
7 %	78	75
8 %	68	65
9 %	47	45
10 %	42	40
11 %	38	36
12 %	35	33
13 %	32	30
14 %	21	20
15 %	19	18
16 %	18	17
17 %	17	16
18 %	16	15
19 %	15	14
20 %	14	13
21 %	14	13
22 %	13	12
23 %	12	11
24 %	12	11
25 %	11	10
26 %	11	10
27 %	11	10
28 %	10	9
29 %	10	9
30 %	9	8

Enumeration of *Salmonella*

The material can also be used for testing the enumeration of *Salmonella*. For the determination of number of *Salmonella* colony forming particles in one capsule (*z*) the capsules have to be enumerated according to a strict protocol as described in annex D of the report (Enumeration of *Salmonella* in reference materials for food microbiology). It is essential that the capsules are completely dissolved as otherwise the *z* is underestimated. Based on the certified value, the homogeneity of the batch and the variation between laboratories, 95 % confidence intervals are calculated for the mean *z* that should be found when a laboratory has analysed a certain number of capsules. These intervals are presented in Table 2. When a laboratory for example examined 50 capsules, the mean level of contamination should be between 3.8 and 6.4 *Salmonella* per capsule.

Table 2: Confidence intervals for the mean level of *z* in relation to the number of capsules analysed.

Number of capsules analysed	Interval for mean <i>z</i>
50	3.8 – 6.4
40	3.8 – 6.4
30	3.7 – 6.5
25	3.6 – 6.6
20	3.5 – 6.7
10	3.2 – 7.2

STORAGE

Upon receipt the material has to be stored at $(-20 \pm 5) ^\circ\text{C}$ until reconstitution.

However, the European Commission cannot be held responsible for changes that happen during storage of the material at the customer's premises, especially of opened samples.

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NOTE

A technical report on the production of BCR-507R is available on the internet (<http://www.irmm.jrc.be>). A paper copy can be obtained from IRMM on request.